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(57) Abstract

Synthetic peptides which consist of 5 to 25 amino acids including an amino acid sequence comprising at least IGF-1 amino acids 60-64 wherein amino acid 60 is substituted or derivatized are disclosed. The peptides inhibit IGF-1 induction of autophosphorylation by IGF-1R. Pharmaceutical compositions comprising the peptides together with pharmaceutically acceptable carriers or diluents are disclosed. Methods of inhibiting proliferation of cells that comprise human insulin-like growth factor 1 receptors comprising contacting a proliferating cell and methods of treating an individual suspected of suffering from or susceptible to a disease associated with undesirable cell proliferation are disclosed.

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IGF-1 ANALOGS

BACKGROUND OF THE INVENTION

This application is related to PCT Application Serial Number PCT/US93/04329 filed May 7, 1993 and U.S. Application Serial Number 07/881,524 filed May 8, 1992, which are both incorporated herein by reference.

Evidence indicates that the interaction of Insulin-like Growth Factor-1 (IGF-1) with its own receptor (IGF-1R or, alternatively referred to as type 1 receptor) plays a major role in normal development and in the control of both normal and abnormal cell growth. In growth hormone disturbances of growth as, for instance, in acromegalics and in patients with growth hormone deficiency, clinical assessments of disease activity correlate far better with blood levels of IGF-1 than they do with growth hormone concentrations (Van Wyk et al., *The Biology of Normal Human Growth*, pp. 223-239, Raven Press, NY (1981)). Werner et al., *Proc. Nat. Acad. Sci. USA*, 86:7451-5 (1989) have shown that the mRNA levels for the IGF-1R decrease steadily in all tissues during post-natal development, reaching a maximum during the perinatal stages. IGF-1 mRNA, instead, is not so tightly regulated during development as the mRNA for the IGF-1 R, and actually reaches maximum expression in the adult liver, which is the main site of production of IGF-1.

Apart from these general considerations, a number of reports have appeared indicating that the interaction of IGF-1 with its own receptor play a major role in cell growth. For instance, IGF-1Rs are present in phytohemagglutinin activated T lymphocytes, Kozak et al., *Cell Immunol.*, 1009:318-331 (1987) and in K562 cells that are a human erythroleukemia cell line,

Hizuka et al., *Endocrinol. Japon*, 34:81-88 (1987). In fact, K562 cells grow vigorously in serum free media (SFM) containing only IGF-1 or supraphysiological concentrations of insulin. An abundance of IGF-1Rs has also been reported in lymphoblasts of human T cell leukemias, Lee et al., *J. Clin. Endocrinol. & Metabol.*, 62:28-35 (1986), and in HL60 cells, Pepe et al., *J. Cell Physiol.*, 133:219-227 (1987). In our own laboratory, we have been able to show that the mRNA for the IGF-1R is over-expressed in HL60 cells. HL60 cells, as well as other cell lines, grow well in serum-free medium containing only insulin in supraphysiological concentrations. In Burkitt cells, the number of IGF-1Rs increase between G_1 and S-3 phase, Hartman et al., *Leukemia*, 2:241-4 (1988). Stem cells and progenitor cells also seem to require IGF-1 for growth. Goldring and Goldring, *Eucar. Gene Express*, 1:-301-326 (1991), list several references indicating that IGF-1 increases the proliferation of keratinocytes, smooth muscle cells, osteoblasts, chrondrocytes and neuronal cells (see their Table 4). The IGF-1R is induced by estrogens in breast cancer cell lines, Stewart et al., *J. Biol. Chem.*, 265:21172-8 (1990), Pekonen et al., *Cancer Res.*, 48:1343-7 (1988), Peyrat et al., *Cancer Res.*, 48:6429-33 (1988), Foekens et al., *Cancer Res,*, 49:5823-8 (1989), and the expression of IGF-1Rs seems to correlate with the growth of breast cancer, at least just as well as the estrogen receptors or the EGF receptor. Other tumors in which an increased expression of IGF-1R or, at least, IGF-1 binding sites, have been reported include small cell lung cancer, Kiefer et al., *Exp. Cell Res.*, 184:396-406 (1989), Minuto et al., *Cancer Res.*, 48:3716-9 (1988), Nakanishi et al., *J. Clin. Invest.*, 82:354-9 (1988), choriocarcinoma cells, Ritvos et al., *Endocrinology*, 122:395-401 (1988), malignant glioma, Gammeltoft et al., *Cancer Res.*, 48:1233-7 (1988), renal carcinoma, Pekonen et al., *Int. J. Cancer*, 43:1029-33 (1989), and neoplastic human endometrium, Talavera et al., *J. Cancer Res.*, 50:3019-24 (1990). A role of the IGF-1R in growth has also been reported in human melanoma cells, Stracke et al., *J. Biol. Chem.*, 264:21544-9 (1989), and in tumors of neural origins like neuroblastomas or

pheochromocytomas, Ota et al., *Molec. Brain Res.*, 6:69-76 (1989) and Ota et al., *Cur. J. Biochem.*, 174:521-30 (1988). However, the best evidence that the IGF-1R plays a major role in the control of cellular proliferation comes from studies with fibroblasts in cell cultures.

When IGF-1 binds to IGF-1R, IGF-1R undergoes autophosphorylation. The autophosphorylation is believed to be an important event in cell growth and proliferation. Thus, IGF-1 induced autophosphorylation of IGF-1R is believed to be involved in the undesirable cell growth and proliferation involved in the pathogenesis associated with diseases and disorders such as, for example, cancer, restenosis and asthma.

There is a need for pharmaceutical compositions which can effectively inhibit the cell proliferation which results from IGF-1R autophosphorylation which normally occurs when IGF-1R bind with IGF-1. There is a need for pharmaceutical compositions which can effectively inhibit the IGF-1 induced autophosphorylation of IGF-1R which normally occurs when IGF-1R binds with IGF-1. There is a need for a method of inhibiting cell proliferation which results from IGF-1R autophosphorylation which normally occurs when IGF-1R bind with IGF-1. There is a need for a method of inhibiting IGF-1 induced autophosphorylation of IGF-1R which normally occurs when IGF-1R binds with IGF-1. There is a need for a method of treating individuals suspected of suffering from or susceptible to diseases and disorders associated with undesirable cell proliferation.

SUMMARY OF INVENTION

The present invention relates to a synthetic peptide consisting of

a) 5 to 25 amino acids, and

b) an amino acid sequence comprising at least IGF-1 amino acids 60-64 wherein amino acid 60 is substituted or derivatized and said peptide inhibits IGF-1 induction of autophosphorylation by IGF-1R.

The present invention relates to pharmaceutical composition comprising:

a) synthetic peptide consisting of

i) 5 to 25 amino acids, and

ii) an amino acid sequence comprising at least IGF-1 amino acids 60-64 wherein amino acid 60 is substituted or derivatized and said peptide inhibits IGF-1 induction of autophosphorylation by IGF-1R; and

b) a pharmaceutically acceptable carrier or diluent.

The present invention relates to methods of inhibiting proliferation of cells that comprise human insulin-like growth factor 1 receptors comprising contacting a proliferating cell with a synthetic peptide consisting of

a) 5 to 25 amino acids, and

b) an amino acid sequence comprising at least IGF-1 amino acids 60-64 wherein amino acid 60 is substituted or derivatized and said peptide inhibits IGF-1 induction of autophosphorylation by IGF-1R.

The present invention relates to methods of treating individuals suspected of suffering from or susceptible to a disease associated with undesirable cell proliferation comprising:

administering to an individual who is suspected of suffering from or who has been identified as being susceptible to a disease associated with undesirable cell proliferations an effective amount of a peptide consisting of

a) 5 to 25 amino acids, and

b) an amino acid sequence comprising at least IGF-1 amino acids 60-64 wherein amino acid 60 is substituted or derivatized and said peptide inhibits IGF-1 induction of autophosphorylation by IGF-1R.

DETAILED DESCRIPTION OF INVENTION

Human IGF-1 is a 70 amino acid protein that consists of 4 principle domains. The first 29 residues of IGF-1 bear a strong resemblance to the B chain of insulin and, consequently,

are known as the B domain. IGF-1 residues 42-62 are homologous to the insulin A chain and hence, known as the A domain. Intervening between the B and A domains (residues 30-41) is the C domain. Finally, the last 7 amino acids (residues 63-70) have been referred to as the D domain. The sequence of IGF-1 is known (SEQ ID NO: 1). Rotwein, P., *et al.* (1986) *J. Biol. Chem.* 261:4828-4832 disclose sequence translated from the DNA sequence. Jansen, M., *et al.* (1983) *Nature* 306:609-611 disclose sequence translated from the mRNA sequence. Met-24 is proposed as a likely initiator. Rinderknecht, E., and Humbel, R.E., (1978) *J. Biol. Chem.* 253:2769-2776 disclose sequence of residues 49-118 which is the mature protein shown as the 70 amino acid sequence SEQ ID NO:2. The numbers referring to domains, A 42-62, B 1-29, C 30-41, and D 63-70, are derived from the mature form of the protein shown in SEQ ID NO:2.

A detailed solution NMR structure of the core of human IGF-1 was recently reported by Cooke, R.M., *et al.* (1991) *Biochem.*, 30:5484-5491. The hydrophobic core of IGF-1 is strikingly similar to insulin. In this light, it is interesting to note that, in addition to binding to IGF-1R, IGF-1 also binds the insulin receptor, albeit with lower affinity (Massague, J. and Czech, M.P., (1982) *J. Biol. Chem.*, 257:5038-5045). The most striking structural differences occur between IGF-1 and an insulin dimer because of the inclusion of the C and D domains in the IGF-1 structure. Both the C and D domains were poorly resolved in the structures due to their intrinsic mobility.

IGF-1 binding to IGF-1R induces autophosphorylation of IGF-1R which activates IGF-1R. Activated IGF-1R is associated with cellular growth and proliferation. In diseases and disorders characterized by undesirable cell growth and proliferation such as, for example, cancer, restenosis and asthma, inhibition of IGF-1R autophosphorylation is desirable as a means of preventing IGF-1R activation. Thus, pharmaceutical compositions useful to treat diseases characterized by cell growth and proliferation are desirable and may include compounds which inhibit the activation of IGF-

1R that occurs when the receptor binds with IGF-1. Such compounds may prevent binding or allow binding but prevent induction of autophosphorylation of IGF-1R by IGF-1.

A molecular model of the human IGF-1 (for general details regarding the building of this molecular model (see, Jameson, B.A., (1989) *Nature*, 341:465-466, which is incorporated herein by reference) that is consistent with the NMR data obtained by Cooke *et al.* (1991) (*supra*) has been developed. In this model, the C and D domains appear as "flaps" which flank the insulin-conserved receptor binding cleft (residues 21-24); Cascieri, M.A., *et al.* (1988) *Biochem.*, 27:3229-3233; Bayne, M.L., *et al.* (1989) *J. Biol. Chem.*, 264:11004-11008. Evidence indicates that these flaps are directly involved in the specific binding to IGF-1R. It has been observed that deletion of the D domain of IGF-1 increased the affinity of the mutant IGF-1 for binding to the insulin receptor, while decreasing its affinity for the IGF-1R (Cascieri *et al.* (1988) *supra*). Furthermore, some or all of the residues within the C domain, which flank the conserved binding cleft in IGF-1, but not in insulin, appear to be required for distinguishing between the IGF-1R and insulin receptors (Bayne *et al.* (1989) *supra*); Cascieri, M.A. and Bayne, M.L., *Molecular and Cellular Biology of IGFs and Their Receptors*, LeRoth, D. and Raizada, M.K., Eds., Plenum Press (London 1990).

Evidence is provided herein showing that the C and D "flaps" of IGF-1 are involved with the highly specific binding of this protein to IGF-1R. Peptides which comprise at least a portion of either flap may be used to inhibit IGF-1 induction of IGF-1R autophosphorylation. Targeting the C and D domains of IGF-1 for synthetic analog design has yielded highly specific competitive inhibitors of IGF-1R binding. In particular, the C domain (residues 30-41) and D domain (residues 63-70) and fragments thereof, alone or linked to IGF-1 and/or non-IGF-1 sequences have been selected for peptide mimicry. Further, analogs which do not inhibit IGF-1/IGF-1R binding at all concentrations may inhibit autophosphorylation

of IGF-1R at some concentrations and are therefore IGF-1 autophosphorylation inhibitors nonetheless.

It has been discovered that peptide analog JB3, which is a circular peptide made up of D amino acids in the reverse order of the sequence 60-69 that occurs in IGF-1 linked by disulfide bonds from terminal cysteines is biphasic, that is JB3 is capable of inhibiting autophosphorylation of IGF-1R when present at low concentrations and capable of stimulating autophosphorylation of IGF-1R when present at high concentrations. It is believed that peptide analogs that comprise amino acids 63 and 64 which are part of the D region as well as amino acid 60, 61 and 62 which are part of the A region are biphasic, that is they are capable of inhibiting autophosphorylation of IGF-1R when present at low concentrations and capable of stimulating autophosphorylation of IGF-1R when present at high concentrations. The IGF-1R stimulating activity is linked to the tyrosine at position 60. It has been discovered that elimination, derivitization or substitution of the tyrosine residue that occurs at position 60 in JB3 eliminates the biphasic activity of such compounds and renders it capable of inhibiting autophosphorylation of IGF-1R when present at high as well as low concentrations. It has been observed that iodination of the tyrosine that occurs at position 60 or substitution of the tyrosine at position 60 with a different amino acid, particularly leucine, eliminates the biphasic activity.

According to the present invention, in analogs modelled upon the D region that comprise amino acids 63 and 64 and which also include amino acids 60 and preferably 60-62 of the A region, the tyrosine that occurs at position 60 is eliminated, derivatized or substituted; preferably, the tyrosine is substituted with leucine or phenylalanine, most preferably leucine. In such embodiments, the analogs exhibit inhibition of autophosphorylation of IGF-1R without biphasic activity. Elimination of tyrosine in analogs at the position that corresponds to residue 60 of IGF-1 eliminates the observed biphasic activity observed. According to some embodiments, the

tyrosine is preferably derivatized to change the conformation of the peptide or physical structure of the residue, such as by iodination of the tyrosine. According to preferred embodiments, the tyrosine is preferably substitute with another amino acid, most preferably leucine.

The methods of the present invention include methods of treating individuals suffering from or susceptible to diseases and disorders characterized by cell proliferation comprising the steps of administering to an individual an effective amount of a peptide which inhibits IGF-1 induced autophosphorylation of IGF-1R. These diseases and disorders include, but are not limited to, restenosis of the coronary arteries after angioplasty, human neoplasia such as cancer of the prostate, tumors in pleural and peritoneal cavities and brain metastases, smooth muscle cell hyperplasia in asthma, burns and wounds, and bone marrow containing highly proliferating cells. Diagnosis of diseases and disorders involving undesirable proliferation of cells such as those diseases and disorders outlined above may be made those having ordinary skill in the art. Likewise, identification of those individuals susceptible to such diseases and disorders is also within the routine ability of those having ordinary skill in the art. For example, methods of diagnosing cancer and asthma are well known. In the case of a method of preventing restenosis, an individual to whom angioplasty is to be performed may be treated. Pharmaceutical compositions useful in the methods of the present invention are defined below.

Peptides of less than 25 amino acids are provided comprising an amino acid sequence corresponding to at least a portion of the D domain of the human insulin-like growth factor 1 including amino acids 63 and 64 of the D domain and amino acid 60 and preferably amino acids 60-62 of the A region in which the tyrosine that occurs at position 60 is eliminated, derivatized or substituted; preferably, substituted with leucine or phenylalanine, most preferably leucine. These peptides have a restricted conformation and the ability to inhibit the induction of IGF-1R autophosphorylation by natural

IGF-1, thereby inhibiting the action of the IGF-1R. The inhibition of autophosphorylation of IGF-1R can be used as the mechanism to prevent or treat diseases and disorders associated with undesirable and/or abnormal growth and proliferation of cells.

The present invention provides synthetic peptides which are capable of inhibiting the induction of IGF-1R autophosphorylation by natural IGF-1 and thereby cell proliferation and which have less than 25 amino acids which comprise at least a portion of the D domain of IGF-1 including amino acids 63 and 64 of the D domain and amino acid 60 of the A region in which the tyrosine that occurs at position 60 is eliminated, derivatized or substituted. These synthetic peptides are also interchangeable referred to herein as IGF-1 analogs, synthetic analogs or analogs. The present invention provides pharmaceutical compositions that comprise the synthetic peptides of the invention and a pharmaceutically acceptable carrier or diluent.

According to preferred embodiments, the peptides comprise amino acids 60-62 of the A region as well, thus comprising the sequence 60-65 wherein amino acid 60 of the A region in which the tyrosine that occurs at position 60 is eliminated, derivatized or substituted; preferably, substituted with leucine or phenylalanine, most preferably leucine.

Using the amino acid sequence of IGF-1, and the molecular modelling described above, we have synthesized short peptides which function as analogs of IGF-1. The peptides are non-toxic, i.e., the cells exposed to it remain viable for long periods of time. The inhibitor effect is also reversible, i.e., when the peptides are removed and growth factors are added again, the cells resume proliferation. Inhibition is close to 100%, and it should apply to all cells that require the IGF-1/IGF-1 receptor interaction for growth. These cells include the following: fibroblasts, smooth muscle cells, chondrocytes and osteoblasts, hemopoietic cells of various lineages and keratinocytes. Several of these cell types have been actually tested, and the inhibition by the IGF-1 analog is

efficient (almost 100%), and reproducible. For instance, when using the IGF-1 analog, we have inhibited the growth of fibroblasts, and fibroblast-like cells, of T-lymphocytes and of epithelial cells derived from carcinoma of the prostate.

Initial synthetic analogs were designed to incorporate at least a portion of the amino acid sequence of the C and D domains of IGF-1. An attempt was made to maintain the distance geometries and torsional properties of the initial dihedral angles of the domains as they "bud-off" of the hydrophobic protein core. Experimental evidence as well as theoretical calculations indicate strong conformational flexibility of these domains. In order to maximize the overlap between the conformational repertoire of the native protein with that of the synthetic analogs, the analogs have been circularized via an artificially introduced disulfide bridge. With these restraints, the rest of the amino acid sequence of the domain should adopt a folding pattern similar to that imparted by the native structure.

The present invention provides synthetic peptides that are less than 25 amino acids and comprise amino acids 60-65 of IGF-1 in which the tyrosine at 60 is substituted or derivatized. It is preferred that peptides are as small as possible. In some embodiments, the peptides are about 5-20 amino acids. In some embodiments, the peptides are about 5-12 amino acids.

The present invention provides synthetic peptides which contain at least a portion of the IGF-1 D domain of IGF-1 and a portion of the A domain. The portion of the D domain of IGF-1 may be from 2 amino acids to the complete domain (a complete D domain is 7 amino acids). Non-IGF-1 amino acid sequences are provided in some embodiments. In other embodiments, the peptide contains only IGF-1 amino acid sequences. At least 50% of the amino acid sequence of the peptides of the present invention are preferably derived from the portion of the A and D domain of IGF-1 in some embodiments of the invention. In some embodiments which comprise more than two IGF-1 derived amino acid residue sequences, it is preferred

that greater than about 20-25% of the amino acid sequence of the peptides of the present invention are preferably derived from the portion of the A and D domain of IGF-1, more preferably 30-40% and more preferably greater than 50%. In some embodiments, the percentage of amino acid sequence of the peptides of the present invention derived from the portion of the A and D domain of IGF-1 approaches about 60% or about 75% or more.

The present invention provides synthetic peptides which are capable of inhibiting the induction of IGF-1R autophosphorylation by natural IGF-1 and thereby cell proliferation. Example 1 contains a cell proliferation assay which can be used by one having ordinary skill in the art to test whether a peptide has cell proliferation inhibitory activity. Induction of IGF-1R autophosphorylation and inhibition of induction may be carried out essentially by the method of Lammers et al. (1989) *EMBO J.* **8**:1369-1375, which is incorporated herein by reference, using the monoclonal antibody to IGF-1R (Oncogene Sciences, Uniondale, NY), an anti-phosphotyrosine antibody (UBI, Saranac Lake, NY) and the advanced chemiluminescence detection system (Amersham, Arlington Heights, IL).

The peptides of the present invention may be prepared by any of the following known techniques. Conveniently, the peptides may be prepared using the solid-phase synthetic technique initially described in Merrifield (1963) *J. Am. Chem. Soc.* **15**:2149-2154. Other peptide synthesis techniques may be found, for example, in M. Bodanszky *et al.*, *Peptide Synthesis*, John Wiley & Sons, 2d Ed. (1976); Kent and Clark-Lewis in *Synthetic Peptides in Biology and Medicine*, p. 295-358, eds. Alitalo, K., Partanen, P. and Vakeri, A., Elsevier Science Publishers, (Amsterdam, 1985); as well as other reference works known to those skilled in the art. A summary of peptide synthesis techniques may be found in J. Stuart and J.D. Young, *Solid Phase Peptide Synthelia*, Pierce Chemical Company, Rockford, IL (1984). The synthesis of peptides by solution methods may also be used, as described in *The Proteins*, Vol.

II, 3d Ed., p. 105-237, Neurath, H. et al., Eds., Academic Press, New York, NY (1976). Appropriate protective groups for use in such syntheses will be found in the above texts, as well as in J.F.W. McOmie, *Protective Groups in Organic Chemistry*, Plenum Press, New York, NY (1973).

In general, these synthetic methods involve the sequential addition of one or more amino acid residues or suitable protected amino acid residues to a growing peptide chain. Normally, either the amino or carboxyl group of the first amino acid residue is protected by a suitable, selectively-removable protecting group. A different, selectively removable protecting group is utilized for amino acids containing a reactive side group, such as lysine.

Using a solid phase synthesis as an example, the protected or derivatized amino acid is attached to an inert solid support through its unprotected carboxyl or amino group. The protecting group of the amino or carboxyl group is then selectively removed and the next amino acid in the sequence having the complementary (amino or carboxyl) group suitably protected is admixed and reacted with the residue already attached to the solid support. The protecting group of the amino or carboxyl group is then removed from this newly added amino acid residue, and the next amino acid (suitably protected) is then added, and so forth. After all the desired amino acids have been linked in the proper sequence, any remaining terminal and side group protecting groups (and solid support) are removed sequentially or concurrently, to provide the final peptide. The peptide of the invention are preferably devoid of benzylated or methylbenzylated amino acids. Such protecting group moieties may be used in the course of synthesis, but they are removed before the peptides are used. Additional reactions may be necessary, as described elsewhere, to form intramolecular linkages to restrain conformation.

The present peptides may also be prepared by recombinant DNA techniques, although such methods are not preferred because of the need for purification and subsequent

chemical modifications to conformationally restrain the peptides.

In addition to peptides which comprise L amino acids, pharmaceutical compositions according to the present invention may comprise peptides made up of D amino acids. Because most enzymes involved in degradation recognize a tetrahedral alpha-carbon, the D-amino acids were utilized in order to avoid enzyme recognition and subsequent cleavage. Our computer studies indicate that the same folded presentation of the peptide is accomplished by reversing the amino acid sequence, employing D-amino acids. Thus, peptides comprised of D amino acids are less susceptible to degradation.

Conservative substitutions in the amino acid sequence may be made. Those having ordinary skill in the art can readily design IGF-1 analogs with conservative substitutions for amino acids. For example, following what are referred to as Dayhof's rules for amino acid substitution (Dayhof, M.D. (1978) *Nat. Biomed. Res. Found.*, Washington, D.C. Vol. 5, supp. 3), amino acid residues in a peptide sequence may be substituted with comparable amino acid residues. Such substitutions are well known and are based the upon charge and structural characteristics of each amino acid.

The synthetic peptides of the present invention are designed using IGF-1 sequence (SEQ ID NO:1 and SEQ ID NO:2) information, particularly sequences which include the sequence from the A (60-62) and D (63-64) domains. Peptides of less than 25 amino acids total which comprise at amino acid residues 60-64 in which the amino acid at 60 is substituted or derivatized are synthesized. In peptides having 5 amino acid residue sequences that are portions of the A and D domain, the peptide is preferably 7-10 amino acids total. L or D amino acids may be used in the synthesis. Peptides may be synthesized with amino acid sequences in the order they occur in IGF-1 or in the reverse order. In peptides comprising all L amino acids, it is preferred that they are synthesized such that the amino acid sequences are assembled in the order that they occur in IGF-1. In peptides comprising all D amino acids,

it is preferred that they are synthesized such that the amino acid sequences are assembled in the reverse order that they occur in IGF-1.

Synthesized peptides may be circularized in order to mimic the geometry of those portions as they occur in IGF-1. Circularization may be facilitated by disulfide bridges between cysteine residues. Cysteine residues may be included in positions on the peptide which flank the portions of the peptide which are derived from IGF-1. Cysteine residues within the portion of the peptide derived from IGF-1 may be deleted and/or conservatively substituted to eliminate the formation of disulfide bridges involving such residues. Alternatively, the peptides may be circularized by means of covalent bonds, such as amide bonds, between amino acid residues of the peptide such as those at or near the amino and carboxy termini.

According to some embodiments of the present invention, in analogs modelled upon the D region that comprise amino acids 63 and 64 and which also include amino acids 60-62 of the A region, the tyrosine that occurs at position 60 is eliminated, derivatized or substituted; preferably, the tyrosine is substituted with leucine or phenylalanine, most preferably leucine. In such embodiments, the analogs exhibit inhibition of autophosphorylation of IGF-1R without biphasic activity. Elimination of tyrosine in analogs at the position that corresponds to residue 60 of IGF-1 eliminates the observed biphasic activity observed. According to some embodiments, the tyrosine is preferably derivatized to change the conformation of the peptide or physical structure of the residue, such as by iodination of the tyrosine. According to preferred embodiments, the tyrosine is preferably substitute with another amino acid, most preferably leucine.

Peptides in some embodiments consists of SEQ ID NO:5. Peptides in some embodiments comprise SEQ ID NO:5. Peptides in some embodiments consists of fragments of SEQ ID NO:5. Peptides in some embodiments comprise fragments of SEQ ID NO:5.

Peptides for use in pharmaceutical compositions of the present invention may be designed following the guidelines set

out herein and using well known processes. Methods of synthesizing peptides and circularizing them may be performed routinely using standard techniques and readily available starting materials.

To determine whether a peptide having the structural properties defined herein is useful in the pharmaceutical compositions and methods of the present invention, routine assays may be performed using such peptides to determine whether the peptides possess the requisite activity; i.e. whether the peptide can inhibit IGF-1 induced autophosphorylation of IGF-1R. The peptides ability to inhibit cell proliferation may be determined by observing its activity in a cell proliferation assay. As noted above, induction of IGF-1R autophosphorylation and inhibition of induction may be carried out essentially by the method of Lammers et al. (1989) *EMBO J.* **8**:1369-1375, which is incorporated herein by reference, using the monoclonal antibody to IGF-1R (Oncogene Sciences, Uniondale, NY), an anti-phosphotyrosine antibody (UBI, Saranac Lake, NY) and the advanced chemiluminescence detection system (Amersham, Arlington Heights, IL). Example 1 contains a cell proliferation assay which can be used by one having ordinary skill in the art to test whether a peptide has cell proliferation inhibitory activity.

Accordingly, peptides having the structural characteristics described above may be synthesized routinely. Such peptides may be tested using standard assays to determine if they can be used in pharmaceutical compositions and methods according to the present invention.

The present invention relates to pharmaceutical compositions which comprise a peptide of the invention and a pharmaceutically acceptable carrier or diluent.

The peptides of the invention may be used in a method of inhibiting proliferation of cells that comprise human insulin-like growth factor 1 receptors. Such a method comprises contacting a proliferating cell with a peptide of the invention.

The peptides of the invention may be used in a method of treating an individual suspected of suffering from or susceptible to a disease associated with undesirable cell proliferation. Such a method comprises administering to such an individual, an effective amount of a peptide of the invention.

The pharmaceutical composition of the present invention may be formulated by one having ordinary skill in the art with compositions selected depending upon the chosen mode of administration. Suitable pharmaceutical carriers are described in *Remington's Pharmaceutical Sciences*, A. Osol, a standard reference text in this field.

For parenteral administration, the IGF-1 analog can be, for example, formulated as a solution, suspension, emulsion or lyophilized powder in association with a pharmaceutically acceptable parenteral vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils may also be used. The vehicle or lyophilized powder may contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by commonly used techniques. For example, a parenteral composition suitable for administration by injection is prepared by dissolving 1.5% by weight of active ingredient in 0.9% sodium chloride solution.

The pharmaceutical compositions according to the present invention may be administered as a single dose or in multiple doses. The pharmaceutical compositions of the present invention may be administered either as individual therapeutic agents or in combination with other therapeutic agents. The treatments of the present invention may be combined with conventional therapies, which may be administered sequentially or simultaneously.

The pharmaceutical compositions of the present invention may be administered by any means that enables the active agent to reach the targeted cells. Because peptides are

subject to being digested when administered orally, parenteral administration, i.e., intravenous, subcutaneous, intramuscular, would ordinarily be used to optimize absorption. Intravenous administration may be accomplished with the aid of an infusion pump. The pharmaceutical compositions of the present invention may be formulated as an emulsion. Alternatively, they may be formulated as aerosol medicaments for intranasal or inhalation administration. In some cases, topical administration may be desirable.

The dosage administered varies depending upon factors such as: pharmacodynamic characteristics; its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms; kind of concurrent treatment; and frequency of treatment. Usually, the dosage of peptide can be about 1 to 3000 milligrams per 50 kilograms of body weight; preferably 10 to 1000 milligrams per 50 kilograms of body weight; more preferably 25 to 800 milligrams per 50 kilograms of body weight. Ordinarily 8 to 800 milligrams are administered to an individual per day in divided doses 1 to 6 times a day or in sustained release form is effective to obtain desired results.

Depending upon the disease or disorder to be treated, the pharmaceutical compositions of the present invention may be formulated and administered to most effectively inhibit undesirable cell proliferation.

Restenosis is a side effect often occurring when balloon angioplasty is performed. The disruption of the endothelial lining of the blood vessel being cleared by angioplasty exposes the underlying smooth muscle cells. Undesirable smooth muscle cell proliferation occurs when the exposed smooth muscle cells proliferate absent the contact inhibition that occurs when the endothelial lining is present. The proliferating cells congest the blood vessel resulting in restenosis. Much of the undesirable proliferation occurs within the first 24 hours after angioplasty. Thus, when used in conjunction with angioplasty, the pharmaceutical composition is administered one or more times within the first 24 hours

post angioplasty. The pharmaceutical compositions of the present invention may be formulated as an emulsion which can be delivered with the balloon catheter to the area where angioplasty is to take place.

In individuals suffering from asthma, cells of the lungs chronically proliferate and congest the lung. The pharmaceutical compositions of the present invention may be formulated as an aerosol. Such an aerosol medicament may be administered periodically. In individuals suffering from cancer, cells such as tumor cells proliferate chronically. The pharmaceutical compositions of the present invention may be administered periodically. In addition, the pharmaceutical compositions of the present invention may be injected at a site at or near hyperproliferative growth. For example, administration may be by direct injection into a solid tumor mass or in the tissue directly adjacent thereto.

The invention is further illustrated by means of the following, non-limiting examples.

EXAMPLES

Example 1

The following D-amino acid peptide, designated JB3 was synthesized using standard solid phase techniques and D amino acid residues as starting materials. JB3 represents IGF-1 amino acids 60-69 in reverse order with cysteines at each termini to facilitate cyclization.

D amino acid peptide JB3:

Cys Ser Lys Ala Pro Lys Leu Pro Ala Ala Tyr Cys.

Examination of the computer simulations of the family of structures generated for JB3 indicated that the tyrosine residue (at position 11) mimicked a tryosyl residue (position 24) in the native IGF-1 protein. Mutagenesis studies with IGF-1 have implicated this residue in receptor activation. Because of all the analogs lacking agonistic activity also lacked the tyrosine, it was hypothesized that the hydroxylated aromatic ring of the tyrosine was responsible for the receptor stimulating activity. That is, when present at higher

concentrations, the JB3 has an affinity for the receptor that is normally occupied by the A region which causes stimulating activity. As a test of this hypothesis, JB3 was iodinated. The sphere of hydration of an iodine is very large relative to the size of the ring itself and should prevent the receptor interaction at this position. The iodinated JB3 possessed better potency than JB3 and showed no agonistic activity. The inhibitory ability of the iodinated JB3 is, at least, an order of magnitude better than the unmodified JB3.

Peptides with conservative replacements for the tyrosine residue, such as phenylalanine and leucine were prepared. These new analogs possess potent inhibitory activities without inducing receptor stimulation at higher concentrations. The best activity is observed with the JB3Leu peptide (i.e. JB3 peptide with the tyrosine replaced with a leucine residue).

In this embodiment, D-amino acid peptide JB3 is modified to eliminate biphasic activity. The following D-amino acid peptide, designated JB3Leu, was synthesized using standard solid phase techniques and D amino acid residues as starting materials.

D amino acid peptide JB3Leu:

Cys Ser Lys Ala Pro Lys Leu Pro Ala Ala Leu Cys.

This peptide did not stimulate IGF-1R even when present at high concentrations.

Example 2

A pharmaceutical composition is formulated which includes the D amino acid peptide JB3Leu (described above) in phosphate buffered saline. An 800 milligram dose of peptide in this composition is delivered with an infusion pump to an individual within 24 hours after balloon angioplasty is performed.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Bradford A. Jameson
Renato Baserga

(ii) TITLE OF INVENTION: IGF-1 Analogs

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY: Philadelphia
(D) STATE: PA
(E) COUNTRY: USA
(F) ZIP: 19103

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: DISKETTE, 3.5 INCH, 1.44 Mb STORAGE
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: PC-DOS
(D) SOFTWARE: WORDPERFECT

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US/08/167,653
(B) FILING DATE: 15-DEC-1993

(viii) ATTORNEY/AGENT INFORMATION:
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(B) REGISTRATION NUMBER: 33,229
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 153
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Met Gly Lys Ile Ser Ser Leu Pro Thr Gln Leu Phe Lys Cys Cys Phe
1               5                  10                  15

Cys Asp Phe Leu Lys Val Lys Met His Thr Met Ser Ser Ser His Leu
            20                  25                  30

Phe Tyr Leu Ala Leu Cys Leu Leu Thr Phe Thr Ser Ser Ala Thr Ala
        35                  40                  45

Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
    50                  55                  60

Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
65                  70                  75                  80
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Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
                85                  90                  95

Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
            100                 105                 110

Lys Pro Ala Lys Ser Ala Arg Ser Val Arg Ala Gln Arg His Thr Asp
        115                 120                 125

Met Pro Lys Thr Gln Lys Glu Val His Leu Lys Asn Ala Ser Arg Gly
    130                 135                 140

Ser Ala Gly Asn Lys Asn Tyr Arg Met
145                 150
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
1               5                   10                  15

Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
            20                  25                  30

Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
        35                  40                  45

Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
    50                  55                  60

Lys Pro Ala Lys Ser Ala
65                  70
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CLAIMS

What is claimed is:

1. A synthetic peptide consisting of

a) 5 to 25 amino acids, and

b) an amino acid sequence comprising at least IGF-1 amino acids 60-64 wherein amino acid 60 is substituted or derivatized and said peptide inhibits IGF-1 induction of autophosphorylation by IGF-1R.

2. The peptide of claim 1 wherein said peptide comprises a sequence of at least 7 amino acid residues.

3. The peptide of claim 1 wherein at least 50% of amino acid residues of said peptide consists of a sequence corresponding to human insulin-like growth factor 1 sequences.

4. The peptide of claim 1 wherein said peptide comprises at least one D amino acid residue.

5. The peptide of claim 1 wherein said peptide is cyclicized.

6. The peptide of claim 1 wherein amino acid 60 is iodinated tyrosine.

7. The peptide of claim 1 wherein amino acid 60 is leucine or phenylalanine.

8. The peptide of claim 1 wherein amino acid 60 is leucine.

9. A pharmaceutical composition comprising:

a) synthetic peptide consisting of

i) 5 to 25 amino acids, and

ii) an amino acid sequence comprising at least IGF-1 amino acids 60-64 wherein amino acid 60 is

substituted or derivatized and said peptide inhibits IGF-1 induction of autophosphorylation by IGF-1R; and

b) a pharmaceutically acceptable carrier or diluent.

10. The pharmaceutical composition of claim 9 wherein said peptide comprises a sequence of at least 7 amino acid residues.

11. The pharmaceutical composition of claim 9 wherein at least 50% of amino acid residues of said peptide consists of a sequence corresponding to human insulin-like growth factor 1 sequences.

12. The pharmaceutical composition of claim 9 wherein said peptide comprises at least one D amino acid residue.

13. The pharmaceutical composition of claim 9 wherein said peptide is cyclicized.

14. The pharmaceutical composition of claim 9 wherein amino acid 60 is iodinated tyrosine.

15. The pharmaceutical composition of claim 9 wherein amino acid 60 is leucine or phenylalanine.

16. The pharmaceutical composition of claim 9 wherein amino acid 60 is leucine.

17. A method of inhibiting proliferation of cells that comprise human insulin-like growth factor 1 receptors comprising contacting a proliferating cell with a synthetic peptide consisting of

a) 5 to 25 amino acids, and

b) an amino acid sequence comprising at least IGF-1 amino acids 60-64 wherein amino acid 60 is substituted

or derivatized and said peptide inhibits IGF-1 induction of autophosphorylation by IGF-1R.

18. The method of claim 17 wherein said peptide comprises a sequence of at least 7 amino acid residues.

19. The method of claim 17 wherein at least 50% of amino acid residues of said peptide consists of a sequence corresponding to human insulin-like growth factor 1 sequences.

20. The method of claim 17 wherein said peptide comprises at least one D amino acid residue.

21. The method of claim 17 wherein said peptide is cyclicized.

22. The method of claim 17 wherein amino acid 60 is iodinated tyrosine.

23. The method of claim 17 wherein amino acid 60 is leucine or phenylalanine.

24. The method of claim 17 wherein amino acid 60 is leucine.

25. A method of treating an individual suspected of suffering from or susceptible to a disease associated with undesirable cell proliferation comprising:

administering an effective amount of a peptide consisting of

a) 5 to 25 amino acids, and

b) an amino acid sequence comprising at least IGF-1 amino acids 60-64 wherein amino acid 60 is substituted or derivatized and said peptide inhibits IGF-1 induction of autophosphorylation by IGF-1R.

26. The method of claim 25 wherein said peptide comprises a sequence of at least 7 amino acid residues.

27. The method of claim 25 wherein at least 50% of amino acid residues of said peptide consists of a sequence corresponding to human insulin-like growth factor 1 sequences.

28. The method of claim 25 wherein said peptide comprises at least one D amino acid residue.

29. The method of claim 25 wherein said peptide is cyclicized.

30. The method of claim 25 wherein amino acid 60 is iodinated tyrosine.

31. The method of claim 25 wherein amino acid 60 is leucine or phenylalanine.

32. The method of claim 25 wherein amino acid 60 is leucine.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/14576

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 14/00, 7/00, ; A61K 38/00
US CL : 530\324, 327, 328, 330; 514/12, 14, 15, 18
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530\324, 327, 328, 330; 514/12, 14, 15, 18

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	US, A, 5,093,317, (LEWIS ET AL) 03 March 1992, see entire document.	1-5, 11-15 6-10,16-32
A	The Journal of Biological Chemistry, Volume 253, Number 8, issued 25 April 1978, Rinderknecht and Humbel, "The Amino Acid Sequence of Human Insulin-Like Growth Factor I and Its Structural Homology with Proinsulin" pages 2769-2776.	1-32
A	The Journal of Biological Chemistry, Volume 264, Number 19, issued 05 July 1988, Bayne et al, "The C Region of Human Insulin-like Growth Factor (IGF) I Is Required for High Affinity Binding to the Type 1 IGF Receptor", pages 11004-11008.	1-32

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
17 FEBRUARY 1995	02 MAR 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer SG Marshall Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*